Paired measurements of creatinine and specific gravity after water loading

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Objectives:

The purpose of the study was to gain additional information on the effects of water loading on urine concentration, based on paired measurements of urine creatinine and specific gravity. Criteria for specimen validity testing[1] [2], issued by the Department of Health and Human Services, identified a urine specimen as "substituted" based on creatinine ≤ 5 mg/dL and specific gravity ≤ 1.001 or ≥ 1.020. Specimen validity testing is authorized on those urine specimens collected under federally regulated[3] [4] programs. HHS issued a state-of-the-science paper[5] describing its review and evaluation of over forty research studies which examined clinical data from a variety of studies of human populations including varying biological factors in humans, specific medical conditions which result in serious over hydration, and water loading experiments. All of the scientific literature, particularly evaluation of paired values for creatinine and specific gravity, defines urine meeting the above (substituted) criteria as not being consistent with normal human urine. After its establishment, this definition became a subject of debate. Two of the primary complaints were that the data produced from the forty-odd studies did not contain a reasonable number of the paired creatinine and specific gravity data points, nor were much of the data gathered from female subjects. This study was conducted to gain further confidence in the criteria for the specimen defined as "substituted," by gathering paired data from predominately female participants.

Study Protocol:

Since creatinine production is primarily a function of muscle mass[6], participation in the study was purposely biased toward females. All participants in the study were of reasonable working age (19 – 56). All participants volunteered to consume at least 80 oz. of fluid spread evenly over six consecutive hours. The protocol asked for 40 oz. to be consumed within the first three hours of the six-hour test period. This would be immediately followed by the consumption of at least another 40 oz. in the last three hours of the six-hour test period. Urine specimens were collected at the start of the six-hour test period and at the end of each hour in the test period. Urine specimens were also collected on awakening the morning of the test day and on awakening the morning following the test day (this amounted to a total of nine urine specimens expected from each participant).

Each participant was asked to document the amount and type (water, coffee) of fluid consumed from awakening through completion of the six-hour period, along with the total amount of urine produced in the same time period. Height, weight, age, ethnicity,

eating habits, and medications taken regularly and on the day of the collections were also documented.

Laboratory Analysis:

All urine specimens were sent to a HHS-certified laboratory (Kroll LSI, Gretna, LA) where creatinine and specific gravity were measured. Measurements were completed on all specimens within one to four days after being collected. Creatinine was measured on each specimen on a Hitachi 747 using a modified Jaffé method. Instrument calibration was performed using known standards, with calibration being checked using control materials. The accuracy of the creatinine assay at the critical 5 mg/dL concentration was verified by inclusion of a 4.0 mg/dL creatinine quality control with each batch analysis. Specific gravity was measured on each specimen on a Atago Model UG-1 electronic refractometer, with the instrument calibrated to de-ionized water at 1.000 and verified with quality control samples.

Results:

Fifty-four separate participants, plus two participants repeating the study protocol on a different day, provided a total of 500 urine specimens. 504 specimens were expected, however, three participants did not collect one of the specimens on awakening, and one person was unable to complete the second three hours of drinking per the test protocol. Also, two participants, on request, provided an extra specimen one hour after the end of the six-hour test period. Overall, specimens were obtained from thirteen males and 43 females (the two persons repeating the study protocol were female).

Twelve participants (5 men and 7 women) consumed over one gallon of fluid by the end of their test periods. Two participants were unable to consume the minimum amount of fluid originally intended (total of 80 oz., or approximately 2370 mL, spread evenly over the six hours); the remainder consumed at least the minimum requested.

Results of all participant data are shown in Table 1; all paired data points for creatinine and specific gravity are included in Figure 1.

A high percentage of participants -- 75% of all participants -- were able to produce at least one low or "dilute" specimen during the testing process (see Table 2.). A dilute specimen is defined by HHS criteria as: creatinine < 20 mg/dL and specific gravity < 1.003.

Only 113 out of the 500 specimens (22.6%) made it into the dilute range under these extreme conditions.

Typical hydration profiles are shown in Figures 2 and 3.

None of the specimens were identified as "substituted" based on the aforementioned criteria (see Figure 4.).

Conclusion:

The examination of paired values of creatinine and specific gravity from 500 specimens collected under water loading conditions supports the criteria developed by HHS.

[1] Program Document (PD) 035, September 28, 1998, Notice to the National Laboratory Certification Program (NLCP) Inspectors and HHS Certified Laboratories, "Guidance for Reporting Specimen Validity Test Results."
[2] Program Document (PD) 037, July 28, 1999, Notice to the National Laboratory Certification Program (NLCP) Inspectors and HHS Certified Laboratories, "General Guidance/Criteria for Specimen Validity Testing."
[3] Executive Order 12564, 51FR 32889, September 17, 1986, "Drug-Free Federal Workplace."
[4] Title V - Omnibus Transportation Employee Testing Act of 1991, Public Law 102-143, October 28, 1991.
[5] HHS Division of Workplace Programs, "Evaluation of Scientific Data on Specimen Validity Testing Leading to the Identification and Report of a Specimen as Substituted," February 14, 2000.
[6] Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry, 3rd ed. Philadelphia: WB Sanders, 1999.

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Paired Measurements of Creatinine and Specific Gravity After Water Loading (Table 1.)

Study No.	Eth/ Gen	Age	Wgt (lb.)	BWI	Total Dose (mL)	Hrs.	6-hr. Dose (mL)	Creat. (Low pair)	SG	Time Occur. (hr.)	# Dilute Specs.	Notes: (e.g., next low pair -- creat/SG)	Meds., Diet, Misc. (e.g., Smoker - TBP)
G1	WM	22	185	23.75	2370	6	2370	20.7	1.005	2	0		
G2	HF	25	170	27.44	2385	6	2385	10.7	1.001	4	1		Demulen, tetracycline
G3	WF	26	140	26.45	2469	6	2469	18.4	1.003	5	0		BCP
G4	WF	23	119	20.07	2385	6	2385	8.2	1.002	6	1		BCP; fish, dairy products, eggs
G5	HF	31	175	34.18	2370	6	2370	10.9	1.003	3	0		Zoloft 200 mg/day, acetominophen 1000 mg/day
G6	HM	26	165	23.01	3163	6	3163	19	1.002	3	1		
G7	HF	31	105	19.84	2547	9	2370	6.8	1.001	6	4		
G8	BM	45	145	22.05	2370	6	2370	14.9	1.002	3	1		
G9	HF	28	165	28.32	2370	6	2370	7.1	1.002	3	2	31.7 - 1.006 (4), 14.3 - 1.002 (6)	Triphosil, sudafed
G10	WF	24	140	25.61	3280	6	3280	14	1.003	6	1		
G11	HF	25	190	32.61	3711	6	3711	11.9	1.002	3	1		
G12	WM	31	170	22.43	2885	9	2585	22.5	1.003	8	0		
G13	WF	22	135	21.14	2427	6	2427	8	1.001	4	5	12.5 - 1.002 in hr. 6	
L1	HM	48	170	26.63	2368	6	2368	26.1	1.005	3	0		
L2	WM	30	183	24.14	4850	6	4850	11.7	1.002	3	3		
L3	HF	53	145	24.13	3510	8	3511	7.4	1.002	4	3	20.4 - 1.004 (6), 7.8 - 1.001 (8)	Benadryl w/in 24 hrs., L-Lysinc 500 mg 3x/day
L4	HF	30	120	22.67	3941	7	3546	8.7	1.001	5	5	25.2 - 1.004 (6), 9.5 - 1.001 (7)	
L5	HF	25	130	24.56	4849	7	4494	6.6	1.001	6	4	7.5 - 1.001 (7)	Amoxicillin 500 mg 3x/day
L6	HF	25	95	18.55	2682	6	2287	11.8	1.001	5	5		
L7	WM	25	170	23.06	2457	6	2457	18.8	1.002	5	1		
L8	WM	31	190	28.06	3650	9	2940	9.9	1.002	9	2		Claratin-d, flonase, soma, naproxen
L9	AM	34	180	27.37	3747	6	3747	45.7	1.008	6	0		Actifed, Tavist-D
L10	HF	52	150	28.34	3627	8	3154	7.7	1.001	8	3	8.7 - 1.002 (4), 25.0 - 1.001 (7)	
L11	HM	55	150	24.21	4057	9	3507	10.2	1.002	9	1		Septra
W1	BF	41	214	31.15	2959	13	2367	11.1	1.001	13	1		
W2	WF	48	138	21.61	2423	8	1713	13.5	1.003	4	0	13.6 - 1.003 (8)	
W3	WM	52	172	24.68	4350	13	1880	8.7	1.002	9	1		
W4	WF	41	140	24.03	5100	10	4140	6.9	1.001	10	6		
W5	WF	29	115	19.43	2364	6	2364	8.4	1.002	3	1	10.4 - 1.003 (5)	BCP, Pentasa 4000 mg/day
E1	WM	49	185	23.75	4658	9	4185	17.2	1.003	9	0	working outside	
E2	WM	39	230	31.19	4260	9	3668	20.8	1.003	6	0		
E3	WF	51	150	24.21	4070	9	3570	7.6	1.002	8	3	7.9 - 1.002 (9th hr.)	
E4	WF	27	138	23.69	2366	6	2366	8.2	1.001	2	4	9.0 - 1.001 (3)	
E4-2	WF	27	138	23.69	3252	6	3252	9.3	1.002	5	4	9.5 - 1.001 (6)	
E5	WF	24	130	23.78	2958	6	2958	7.8	1.002	3	2		
E6	WF	41	97	18.94	1728	6	1728	10.1	1.003	6	0		
E7	WF	41	125	23.62	2952	6	2952	7.2	1.001	4	4		
E8	WF	56	135	22.46	3193	6	3193	7.3	1.001	3	5	8.4 - 1.001 (6)	
E9	WF	43	120	21.95	2370	6	2370	12.6	1.002	1	4		
E10	WF	28	210	34.41	2370	6	2370	12.8	1.004	3	0		
E10-2	WF	28	210	34.41	2958	6	2958	11.5	1.002	2	1		
E11	WF	23	103	18.25	2337	6	2337	13.0	1.005	4	2		
E12	BF	28	138	27.87	2622	6	2622	95.0	1.015	3	0		
E13	BF	26	110	17.75	1185	3	1185	42.9	1.009	3	0		
E15	WF	24	125	22.86	2928	6	2928	9.8	1.002	2	2	9.8 - 1.002 (5)	
E16	AF	32	140	24.80	2370	6	2370	27.1	1.008	2	0		
E17	AF	19	90	17.58	1004	6	1004	5.2	1.002	3	1		
E19	WF	49	230	36.02	5146	12	2929	8.2	1.002	12	1	6.7 - 1.003 (12)	
E20	WF	31	180	28.19	5320	9	5202	5.1	1.001	8	5	5.6 - 1.001 (10)	
E21	WF	28	130	23.78	2928	6	2928	12.4	1.002	6	1		
E22	WF	23	125	21.80	2607	9	2370	9.1	1.002	7	1		

E24	WF	27	110	18.88	2437	6	2437	8.1	1.002	4	1		
E26	WF	24	110	18.88	2782	9	2486	10.2	1.001	6	3		
E28	BF	38	136	21.95	2921	9	2448	17.7	1.002	8	3		
E30	WF	36	130	20.98	3195	9	2840	7.9	1.002	7	5	8.6 - 1.002 (9)	
S1	WF	26	165	25.84	4832	9	2740	5.2	1.001	5	8	5.8 - 1.001 (6)	creatinine 22.7 morning of test day

Results of All Data

Table 1.

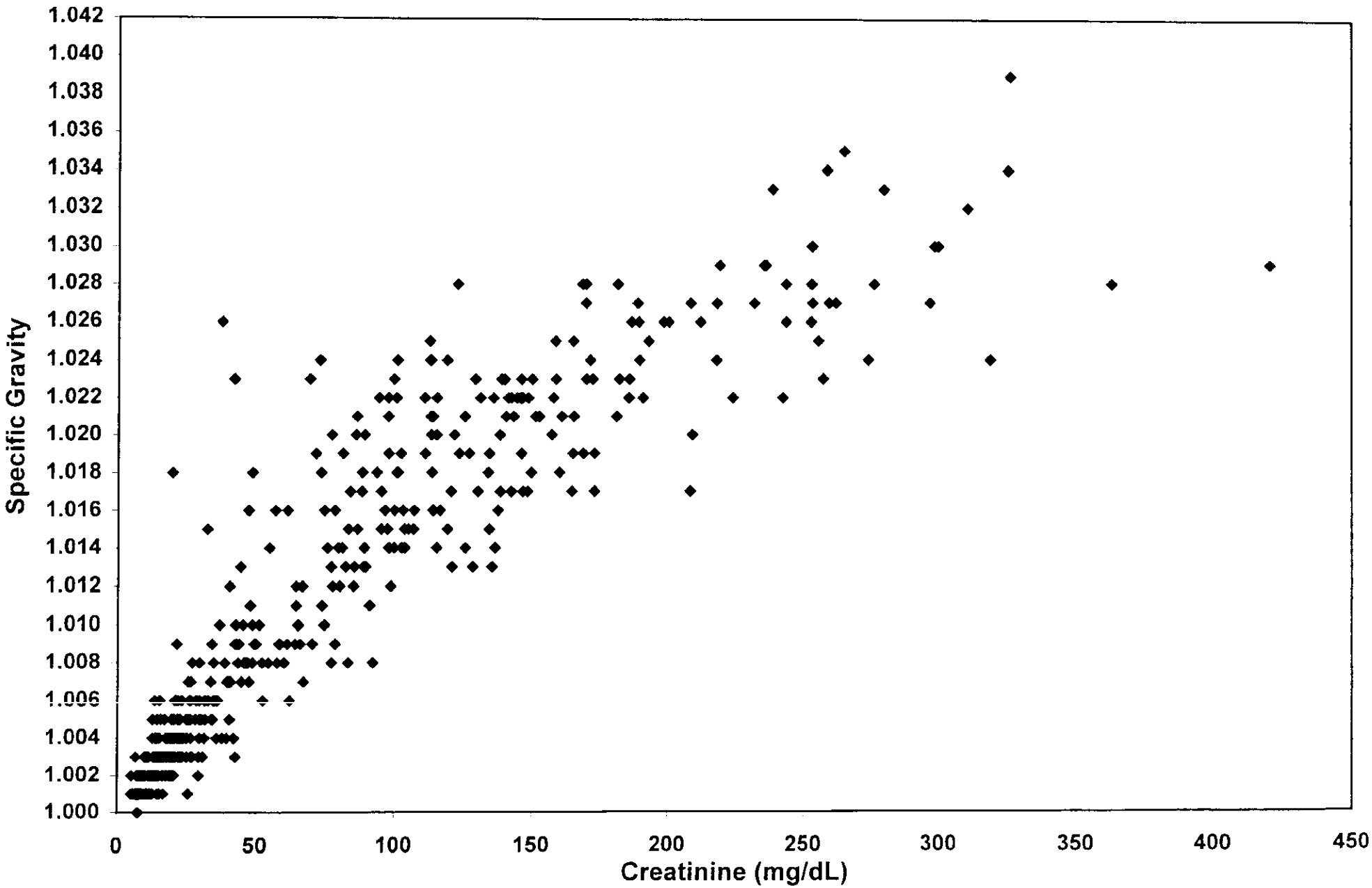

Paired Measurements of Creatinine & Specific Gravity After Water Loading
All data points (500 pairs)
Specific Gravity
1.042
1.040
1.038
1.036
1.034
1.032
1.030
1.028
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
1.000
0
50
100
150
200
250
300
350
400
450
Creatinine (mg/dL)

Figure 1.

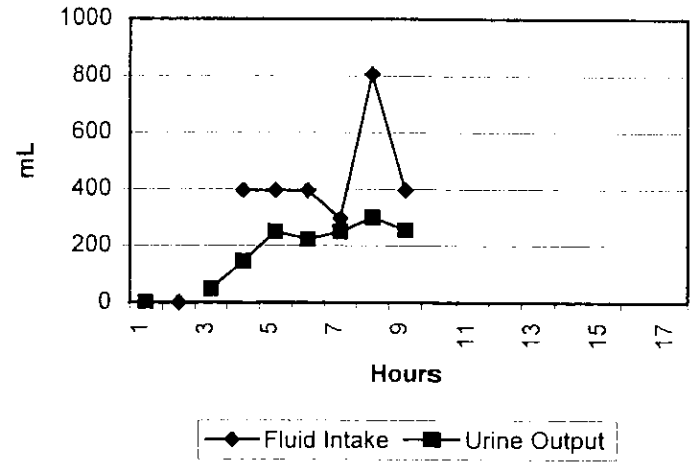
Participants: (Sex -- Race)	Number	Participants with at least one diluted specimen		Number of diluted specimens by race
Male				
White	8	4		7
Black	1	1		1
Hispanic	3	2		2
Asian	1	-		-
Male Sub-Totals	13	7	(53.8%)	10
Female				
While	27	23		70
Black	4	2		4
Hispanic	10	9		28
Asian	2	1		1
Female Sub-Totals	43	35	(81.3%)	103
Participant Totals	56	42	(75%)	113

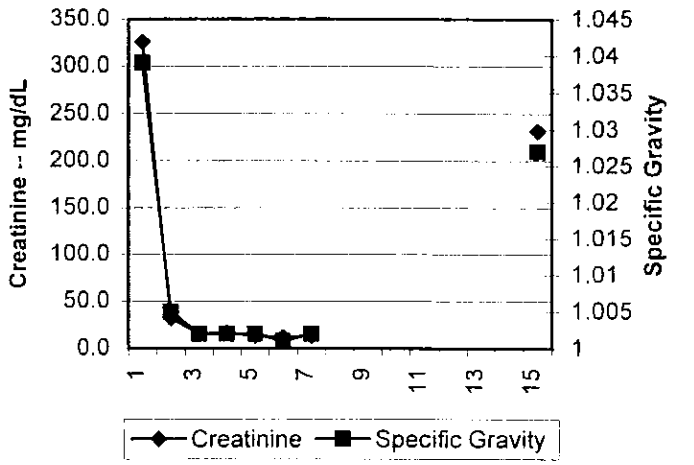
Diluted Specimens by Sex and Race
Table 2.

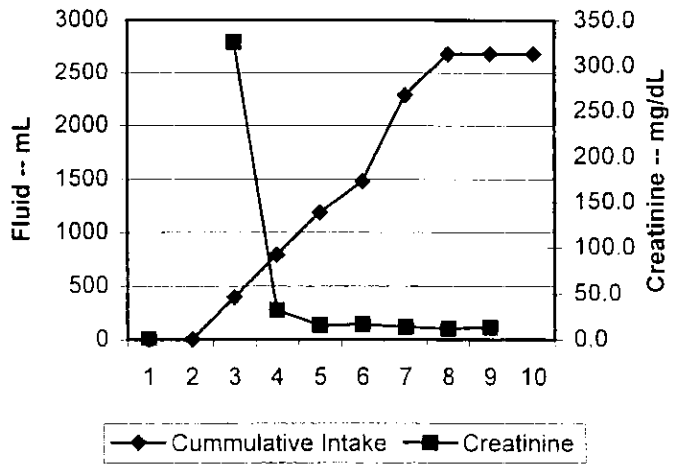
L6		mL				
Time		In	Out	Creatinine	SG	
Awake Day1			0	na	na	
	+1hour	0				
	+2		48	326.0	1.039	
T-hr. 1	+3	395	145	31.7	1.005	
T-hr. 2	+4	395	250	15.3	1.002	
T-hr. 3	+5	395	223	16.4	1.002	
T-hr. 4	+6	295	249	13.5	1.002	
T-hr. 5	+7	807	299	11.8	1.001	
T-hr. 6	+8	395	254	13.4	1.002	
Awake Day2				232.0	1.027	

Age	25
Sex	F
Race	H
Hgt (in.)	60
Wgt (lb.)	95
BMI	18.55

Cumulative Fluid Intake		
Awake		0
	+1hour	0
	+2	395
T-hr. 1	+3	790
T-hr. 2	+4	1185
T-hr. 3	+5	1480
T-hr. 4	+6	2287
T-hr. 5	+7	2682
T-hr. 6	+8	2682
		2682





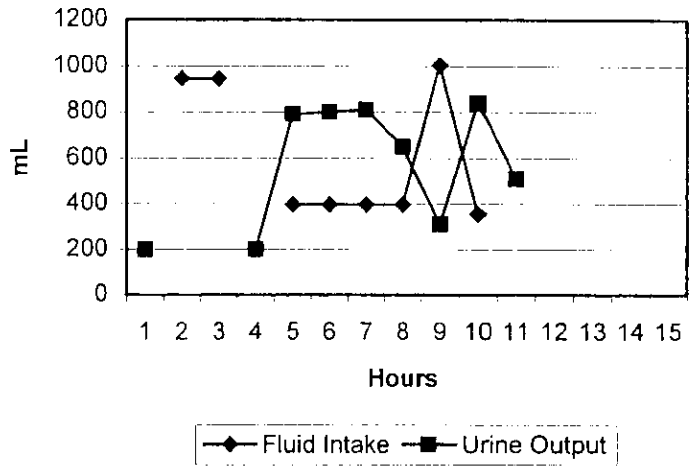


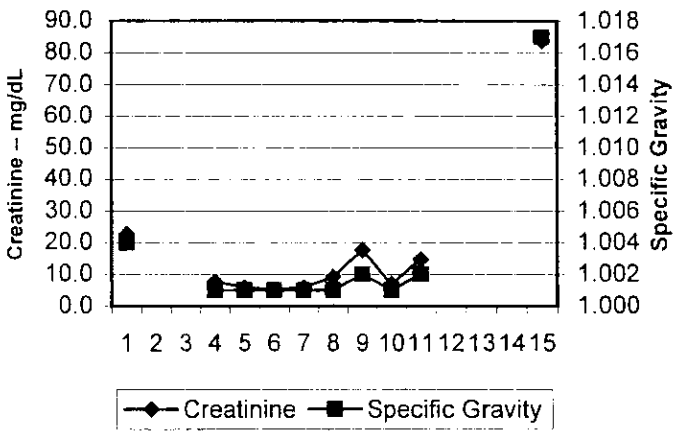
Participant L6
Figure 2.

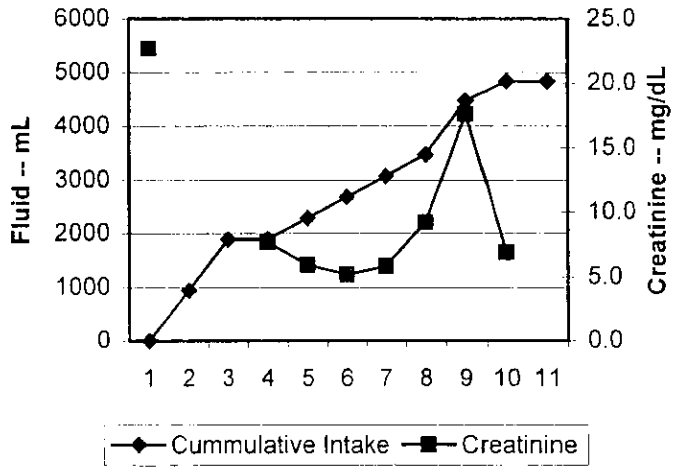
S1		mL			
Time		In	Out	Creatin	SG
Awake Day1			200	22.7	1.004
	+1hour	946			
	+2	946			
	+3		200	7.7	1.001
T-hr. 1	+4	395	790	5.9	1.001
T-hr. 2	+5	395	800	5.2	1.001
T-hr. 3	+6	395	810	5.8	1.001
T-hr. 4	+7	395	650	9.2	1.001
T-hr. 5	+8	1005	310	17.6	1.002
T-hr. 6	+9	355	840	6.9	1.001
T-hr.7	+10		510	14.6	1.002
Awake Day2				83.8	1.017

Age	26
Sex	F
Race	W
Hgt (in.)	67
Wgt (lb.)	165
BMI	25.84

Cumulative Fluid Intake		
Awake		0
	+1hour	946
	+2	1892
	+3	1892
T-hr. 1	+4	2287
T-hr. 2	+5	2682
T-hr. 3	+6	3077
T-hr. 4	+7	3472
T-hr. 5	+8	4477
T-hr. 6	+9	4832
		4832







Participant S1
Figure 3

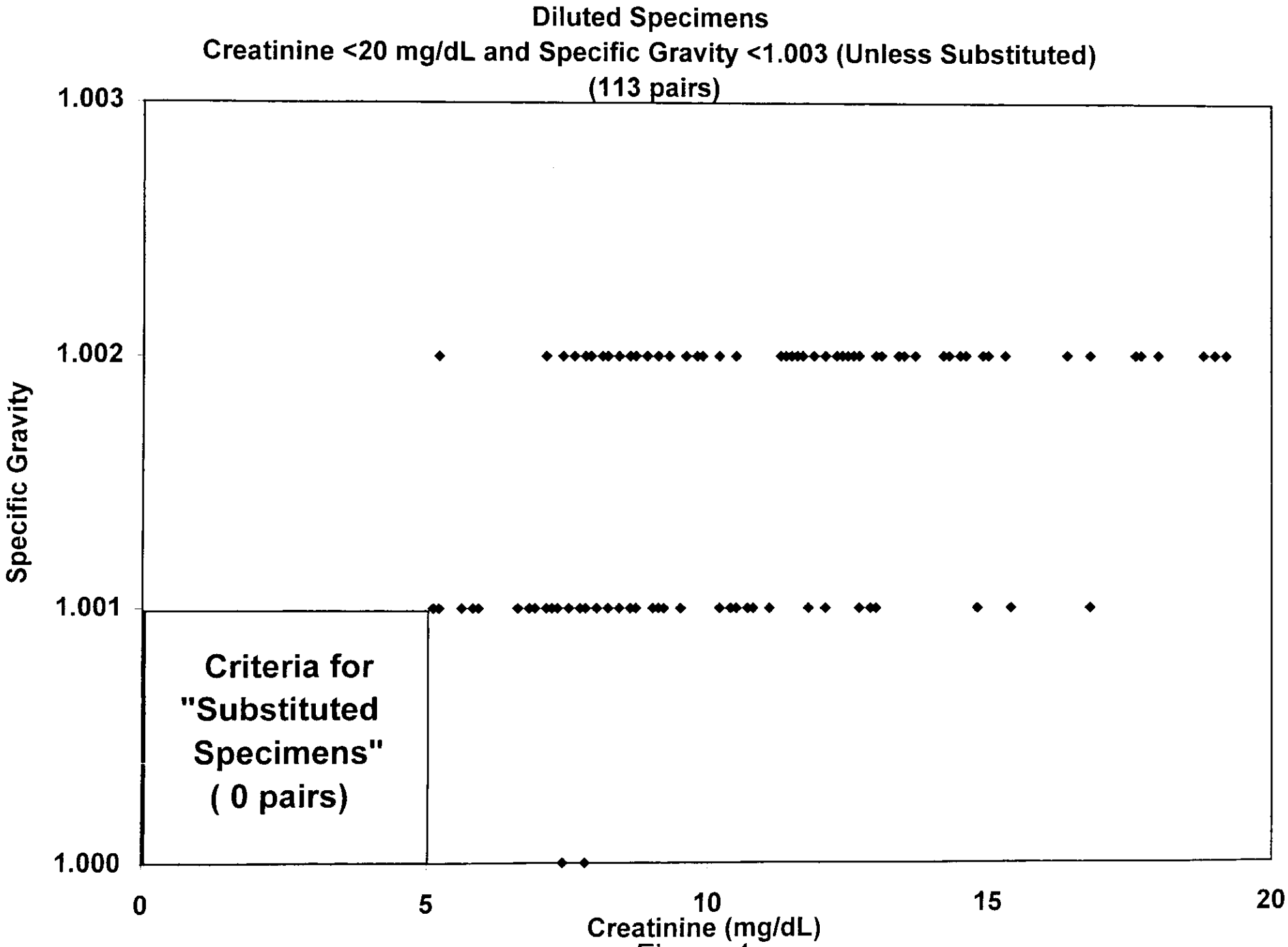

Diluted Specimens
Creatinine <20 mg/dL and Specific Gravity <1.003 (Unless Substituted)
(113 pairs)
1.003
1.002
1.001
1.000
Specific Gravity
Criteria for "Substituted Specimens" (0 pairs)
0
5
10
15
20
Creatinine (mg/dL)

Figure 4.